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TITLE: Voxel-Wise Time-Series Analysis of Quantitative MRI in Relapsing-Remitting MS: Dynamic Imaging Metrics of Disease Activity Including Pre-Lesional Changes

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14. ABSTRACT

Previous MRI studies in MS have retrospectively analyzed normal-appearing brain tissue in locations where typical MS lesions ultimately appeared, finding pre-lesional changes in several MRI metrics. However, studies have not been entirely consistent and the development of a prototypical MS lesion cannot as yet be prospectively predicted. The primary objective of this project is to validate the "preactive" lesion hypothesis in MS by identifying the spatiotemporal imaging signature of white matter destined to undergo acute, focal inflammation and demyelination—specifically, one that will allow reliable, prospective detection of nascent lesions before they appear on conventional (non-quantitative) imaging. The specific aim is to acquire a longitudinal set of quantitative MRI metrics in MS patients and perform a multivariate spatiotemporal analysis of pre-lesional, normal-appearing white matter, seeking spatially clustered interval changes that presage the appearance of a typical MS plaque.

Over the past year, the quantitative MRI protocol has been developed and optimized; enrollment and scanning of subjects is awaiting IRB approval of the study protocol, which is imminent.

15. SUBJECT TERMS

Multiple sclerosis, magnetic resonance imaging, longitudinal studies, preactive lesions

INTRODUCTION: Previous MRI studies in MS have retrospectively analyzed normal-appearing brain tissue in locations where typical MS lesions ultimately appeared, finding pre-lesional changes in several MRI metrics. However, studies have not been entirely consistent and the development of a prototypical MS lesion cannot as yet be prospectively predicted. The primary objective of this project is to validate the "preactive" lesion hypothesis in MS by identifying the spatiotemporal imaging signature of white matter destined to undergo acute, focal inflammation and demyelination—specifically, one that will allow reliable, prospective detection of nascent lesions before they appear on conventional (non-quantitative) imaging. The specific aim is to acquire a longitudinal set of quantitative MRI metrics in MS patients and perform a multivariate spatiotemporal analysis of pre-lesional, normal-appearing white matter, seeking spatially clustered interval changes that presage the appearance of a typical MS plaque.

BODY: We have completed the development and optimization of the quantitative MRI pulse sequences to be used for the project, detailed below and summarized as follows: (1) myelin water mapping based on the mcDESPOT pulse sequence as originally reported by Deoni et al [1] and adapted by our group [2,3]; (2) magnetization transfer (MT) imaging as adapted and optimized by our group [4-7]; and hybrid diffusion imaging (HYDI), developed and optimized by our group [8-10]. Additionally, we developed the post-processing pipeline to be used for these multiparametric images, with key stages including brain extraction, co-registration of images from different modalities and time-points, and segmentation of normal-appearing white matter. Approval of the study protocol has been granted by our own IRB but is still pending from the DOD IRB; therefore we have not yet enrolled or scanned any study subjects. We are ready to begin enrolling and scanning immediately upon receiving IRB approval, which is imminently expected. A one-year no-cost extension to this grant is now in effect.

Myelin Water Mapping: We made several technical improvements to our rapid myelin water imaging technique, mcDESPOT. This technique relies on the collection of multiple SPGR and SSFP rapid steady-state imaging sequences, both of which are highly sensitive to transmit flip angle ($B1$), which may vary up to 30% across an image volume at 3T because of dielectric effects. Additionally, SSFP sequences are sensitive to inhomogeneity in the main magnetic field ($B0$), such as resulting from air/tissue interfaces. Both $B0$ and $B1$ fields change over time as well as with patient position and head geometry. These errors may lead to technical variations in estimated myelin content across scan sessions, confounding efforts to track genuine, pathological, pre-lesion changes over time.

To correct for errors related to $B1$, we invested time developing and optimizing a technique called Actual Flip-angle Imaging [3,5]. Specifically, we have implemented a sequence on our scanner with proper spoiling settings to ensure accuracy and reproducibility of $B1$ maps.

To correct for errors related to $B0$, significant work has been done to improve the estimation of $B0$ within the mcDESPOT algorithm. The original implementation of mcDESPOT attempted to estimate $B0$ simultaneously with the myelin water fraction and other parameters. SSFP images are known to suffer from dark bands near regions of large $B0$ variations. Although multiple phase cycles can be collected to shift these bands to different spatial locations, the data must be correctly weighted to avoid fitting regions of zero signal (noise-only phase cycles). When $B0$ is estimated simultaneously with other parameters, this weighting factor is not known *a priori*, significantly increasing the complexity and computation time required to obtain a good fit. As an

alternative approach, we developed a novel fitting method that can estimate *B0* by making assumptions to fix or simplify other parameters. Instead of including *B0* in the full model, we use a simplified single-component *T2* model. Since many studies have observed that the ratio of *T2* to *T1* is approximately constant across brain tissues (WM/GM), we further constrain the fitting by fixing values of *T2* based on *T1* obtained from DESPOT1. This allows us to use a simplified local optimization algorithm to estimate *B0* instead of a much slower global stochastic method. This provides a *B0* map as well as *a priori* knowledge of signal weighting which can then be supplied to the full mcDESPOT model, speeding up computation time by a factor of 1.5-2.

Magnetization Transfer (MT) Imaging: We developed a novel method for simultaneous calibration of excitation flip angle and MT saturation power [5]; accounting for B1 and flip angle variations is critical for longitudinal stability of MT imaging which, in turn, is required for the tracking of changes that occur in "pre-lesions" over time. Previous studies have relied on either B1 maps or flip angle maps, but not both, to correct both excitation flip and MT saturation power, which may result in significant errors in MT parameters. Our new method enables accounting for both sources of errors and, as a result, yields more accurate MT measures (Fig. 1).
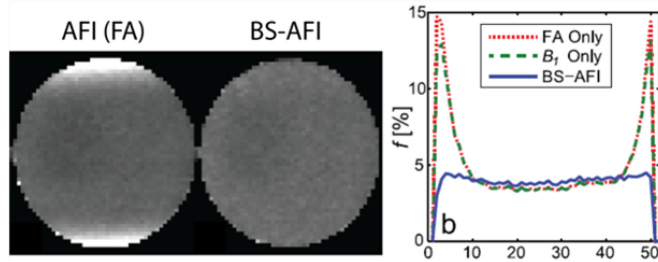

Figure 1. Left: Bound pool fraction *f* in a homogeneous gel phantom calculated using old MT method AFI (FA) and new method (BS-AFI). **Right:** Representative profiles through the images showing the old method overestimating the key MT measure in the areas close to the edges.

We also conceived a modified MT (cross-relaxation) imaging approach that allows advanced modeling of the MT process in tissues [6,7]. This approach increases the accuracy of determination of key MT measures by 10-15% and may increase the pathological specificity of MT imaging for purposes of pre-lesion tissue characterization.

Hybrid Diffusion Imaging: As we are still gaining experience with our novel HYDI diffusion-encoding scheme [8-10], we investigated the diffusion parameters yielded by this scheme (Fig. 2) as a function of age in a cohort of healthy adults, in order to establish normative ranges for these parameters (Table 1), which include measures of the diffusion probability density function (zero displacement probability and mean-squared displacement), biexponential diffusion (i.e., volume fractions of fast/slow diffusion compartments and fast/slow diffusivities), and traditional DTI measures (fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity). The biexponential volume fraction, fast diffusivity, and axial diffusivity were found to be more sensitive to normal aging than the restricted, slow and radial diffusion measures. The biexponential volume fraction showed the most widespread age dependence in a voxel-based analysis although both FA and mean diffusivity did show changes in frontal white matter regions that may be associated with age-related decline.
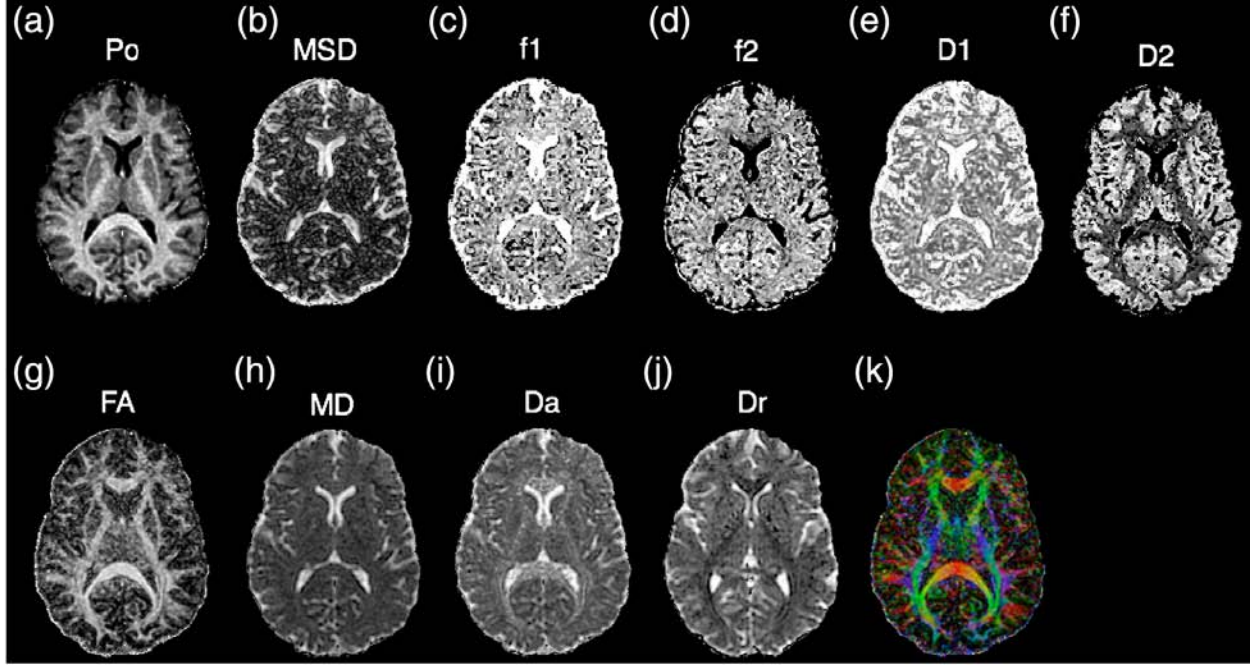
Figure 2. Maps of diffusion measures of a 26-year-old male subject in HYDI experiment. From the whole data set, PDF measures: (a) P0, zero displacement probability, a measure of tissue restriction; (b) MSD, mean squared displacement, a measure of averaged diffusivity. Biexponential model fitting of the whole data set: (c) f1, the volume fraction of the fast diffusion compartment; (d) f2, the volume fraction of the slow diffusion compartment; (e) D1, averaged diffusivity of the fast compartment; (f) D2, averaged diffusivity of the slow compartment. DTI measures from inner shell: (g) FA, fractional anisotropy of diffusion tensor (DT) model; (h) MD, mean diffusivity of DT model; (i) Da, axial diffusivity, (j) Dr, radial diffusivity; and (k) major eigenvector color map.

Table 1. Mean and standard deviation of all HYDI-based diffusion measures in 52 healthy adults for whole-brain white matter and 3D regions of interest.

Tissue		P_0	MSD (10^{-6} s/mm^2)	f_1	f_2	D_1 (10^{-6} s/mm^2)	D_2 (10^{-6} s/mm^2)	FA	MD (10^{-6} s/mm^2)	D_a (10^{-6} s/mm^2)	D_r (10^{-6} s/mm^2)
WBWM	Mean ± SD	0.091 ± 0.005	652 ± 40	0.616 ± 0.065	0.384 ± 0.065	1074 ± 51	185 ± 23	0.506 ± 0.021	459 ± 16	749 ± 24	314 ± 16
CCg	Mean ± SD	0.096 ± 0.008	771 ± 45	0.795 ± 0.027	0.205 ± 0.027	1276 ± 113	88 ± 19	0.771 ± 0.040	578 ± 31	1255 ± 67	239 ± 36
PLIC	Mean ± SD	0.113 ± 0.005	600 ± 34	0.715 ± 0.039	0.285 ± 0.039	1074 ± 73	116 ± 18	0.699 ± 0.28	461 ± 18	914 ± 41	235 ± 20
CCs	Mean ± SD	0.122 ± 0.008	649 ± 44	0.755 ± 0.050	0.245 ± 0.050	1022 ± 133	113 ± 23	0.795 ± 0.039	491 ± 31	1096 ± 54	188 ± 36

Notations:
WBWM: whole-brain white matter.
CCg: genu of corpus callosum.
PLIC: posterior limbs of internal capsule.
CCs: splenium of corpus callosum.
SD: standard deviation across subjects regardless age and gender differences.

Post-Processing Pipeline: We developed a semi-automated post-processing pipeline for the imaging data that incorporates most of the major steps described in the proposal (Figs. 3-5). All imaging data acquired in different orientations (axial and sagittal) are first converted to the NIFTI image format and their orientations changed to axial (orientation of the reference data). Skull stripping (brain extraction) is then performed on high-resolution 3D BRAVO T1-weighted images using the Brain Extraction Tool in the FSL software library (FMRIB, Oxford, UK). To correct intensity variations due to magnetic field inhomogeneity, non-uniformity correction is applied on the extracted brain BRAVO image using JIM analysis software (Xinapse Systems, UK). This reference BRAVO image is then co-registered to the MNI152 template (Montreal Neurological Institute) using the FLIRT (FMRIB Linear Image Registration Tool) brain image registration toolbox in FSL. Eddy current correction is applied to DTI data also using FSL

software. For the next step, all images for a given subject at all scan dates are co-registered to that subject's reference BRAVO series and the BRAVO brain mask generated in the first step is applied to all co-registered images. In addition, non-uniformity correction is applied to high-resolution 3D Cube T2-FLAIR images. Finally, a normal appearing white matter mask is generated using the FMRIB Automated Segmentation Tool (FAST) for four tissue classes (lesion, CSF, normal gray matter, normal-appearing white matter) using multi-channel input (BRAVO T1 and Cube T2 images).
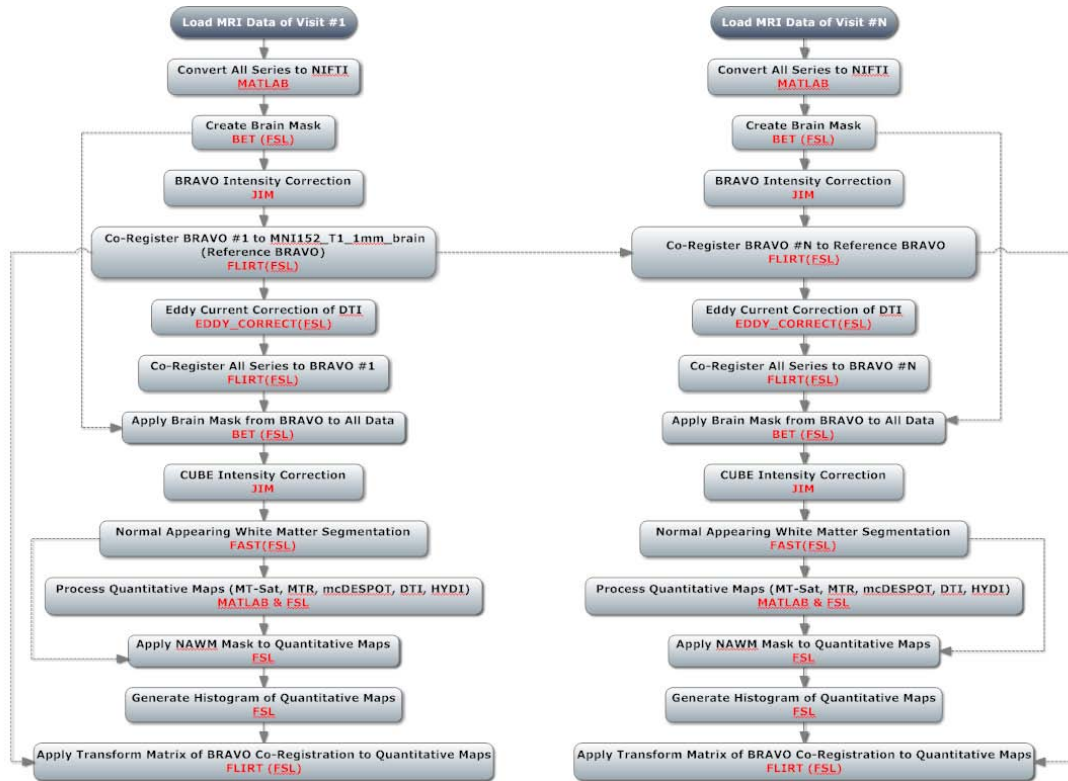

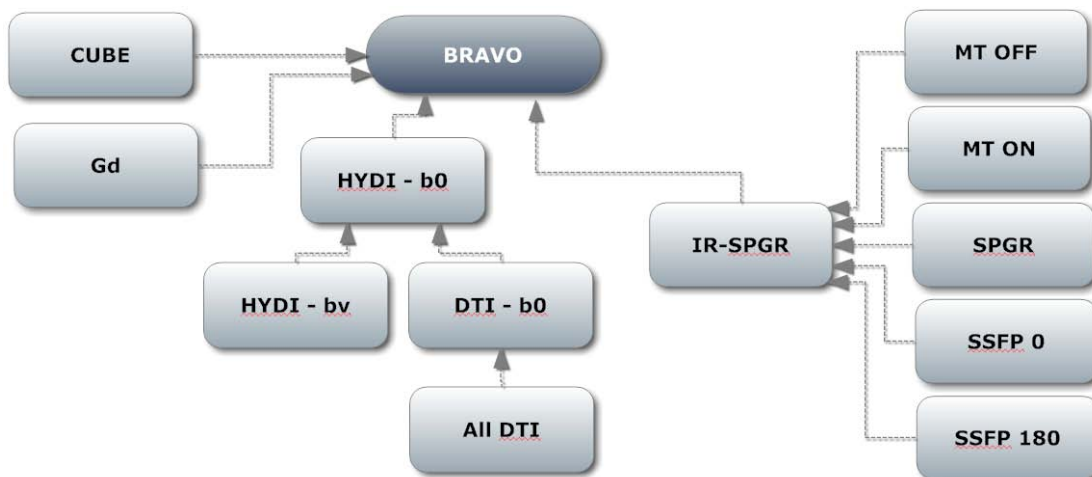**Figure 3:** Overview of post-processing pipeline

Figure 4: Block diagram of image co-registration steps to align quantitative (DTI, MT) with anatomical (T1, T2) images.
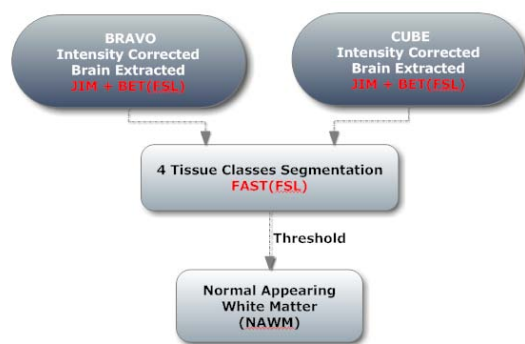
Figure 5: Block diagram of image segmentation to remove lesions and non-white-matter tissues from normal appearing white matter (i.e. potential "pre-lesion" sites).

KEY RESEARCH ACCOMPLISHMENTS: Novel approaches to improve the accuracy and reliability of quantitative MRI (qMRI) targeting cerebral white matter have been developed as detailed above. This work was essential to minimize technical variations in qMRI parameters over time, so that the proposed longitudinal analysis of pre-lesional changes would not be confounded by nuisance sources of variance. We are now ready to begin scanning subjects upon final IRB approval of our study protocol.

REPORTABLE OUTCOMES: None yet, as the study has not yet begun, pending IRB approval as noted above.

CONCLUSION: In summary, we have completed all the preliminary work to begin this study but must await IRB approval before enrolling and scanning subjects.

APPENDICES: Formal study protocol submitted to University of Wisconsin Institutional Review Board is attached.

SUPPORTING DATA:

In addition to the figures and table included above in the body of this report, the following abstracts resulting from this work were very recently accepted for presentation at the next annual meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Melbourne, Australia, May 2012:

1. Hurley SA, Mossahebi P, Johnson KM, Samsonov AA. Simultaneous Mapping of B1 and Flip Angle by Combined Bloch-Siegert, Actual Flip-Angle Imaging (BS-AFI). In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.
2. Mossahebi P, Yarknykh VL, Samsonov AA. Improved Accuracy of Cross-Relaxation Imaging Using On-Resonance MT Effect Correction. In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.

3. Mossahebi P, Samsonov AA. Optimization Strategies for Accurate Quantitative MT Imaging. In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.

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3. Hurley SA, Yarnykh VL, Johnson KM, Field AS, Alexander AL, Samsonov AA. Simultaneous variable flip angle-actual flip angle imaging method for improved accuracy and precision of three-dimensional T1 and B1 measurements. *Magn Reson Med*, in press.
4. Samsonov AA, Alexander AL, Velikina JV, Duncan ID, Field AS. Cross-relaxation imaging of age-related changes in myelin mutant shaking pup. Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 18th Scientific Meeting, Stockholm, Sweden, May 1-7, 2010:4515.
5. Hurley SA, Mossahebi P, Johnson KM, Samsonov AA. Simultaneous Mapping of B1 and Flip Angle by Combined Bloch-Siegert, Actual Flip-Angle Imaging (BS-AFI). In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.
6. Mossahebi P, Yarknykh VL, Samsonov AA. Improved Accuracy of Cross-Relaxation Imaging Using On-Resonance MT Effect Correction. In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.
7. Mossahebi P, Samsonov AA. Optimization Strategies for Accurate Quantitative MT Imaging. In: Proceedings of the International Society for Magnetic Resonance in Medicine (ISMRM) 20th Scientific Meeting, Melbourne, Australia, May 2012.
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10. Wu Y-C, Field AS, Whalen PJ, Alexander AL. Age- and gender-related changes in the normal human brain using hybrid diffusion imaging (HYDI). *NeuroImage* 2011;54(3):1840-1853.